Art Unit: 1641

DETAILED ACTION

1. Applicant's amendment filed 16 November 2007 has been entered.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

> Determining the scope and contents of the prior art.
> Ascertaining the differences between the prior art and the claims at issue.
> Resolving the level of ordinary skill in the pertinent art.
> Considering objective evidence present in the application indicating obviousness or nonobviousness.

2. Claims 1-10, 18 and 308 are rejected under 35 U.S.C. 103(a) as being unpatentable over Burbaum et al. (US 5,981,207) in view of Walker et al. (Signaling pathways underlying eosinophil cell motility revealed by using caged peptides, 1998, PNAS, pg 1568-1573) further in view of Ting et al. (Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells, 2001, PNAS, Vol. 98, no. 26, pages 15003-15008).

Burbaum et al. teach an enzyme (col. 7, lines 44-48; kinase, col. 21, lines 50-57) and a substrate for the enzyme (col. 7, lines 39-43), wherein the substrate is in a first state on which the enzyme can act (col. 7, lines 45-47), thereby converting the substrate to the second state (col. 7, lines 43-47), and a first label, wherein a first signal is exhibited by the first label when the substrate is in the first state and is distinguishable from a second signal

exhibited by the first label when the substrate is in its second state (col. 11, lines 38-41); and one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate and wherein an induced conformational change (release) in the first caging groups permits the enzyme to act upon the substrate(col. 7, lines 39-47). Burbaum et al. fail to teach the enzyme and sensor being in a cell.

Walker et al. teach a caged peptide synthesized outside of a cell and subsequently injected in a cell, in order to provide rapid detection results with good spatial resolution (pg. 1568, right column-left column, first paragraph).

Ting et al. teach a composition comprising: a cell (characterization in mammalian cells, pg. 15003, right column, second paragraph): an enzyme (kinase activity is detected in the cell, therefore the enzyme is in the cell, pg. 15005, right column, cellular response of the Src Reporter) and a sensor, wherein the sensor comprises: a substrate for the enzyme wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state (substrate undergoes significant conformational change, pg. 15003, left column, first paragraph), and a first label, wherein a first signal is exhibited by the first label when the substrate is in its first state and is distinguishable from a second signal exhibited by the first label when the substrate is in its second state (pg. 15003, left column, last paragraph-right column, first paragraph), in order to study kinase and phosphatase functions, localization and activities inside living cells.

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include the sensor of an enzyme of Burbaum et al., in a cell as taught by Walker et al., in order to provide injection into a cell and give time to allow the substrate to distribute evenly so normal cell activity can be detected as taught by Walker et al. and to image localization and activities inside living cells as taught by Ting et al.

Regarding claims 3-5, the claims are drawn to intended use of a composition and do not appear to require any further physical limitations. Therefore, since all physical limitations required for the composition as recited in claim 1 are taught by Burbaum et al. in view of Walker et al. further in view of Ting et al., as described above, the composition of Burbaum et al. in view of Walker et al. further in view of Ting et al. is capable of providing the uses recited in claims 3-5.

With respect to claim 6, Ting et al. also teach the label being an optically detectable label and the second signal being a fluorescent signal that has a greater intensity than the first signal (FRET change creates fluorescent signal after conformational change and reverses the FRET change prior to conformational change, pg. 15003, left column, last paragraph-right column, first paragraph).

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the cell of Burbaum et al. in view of Walker et al., labels and signals as taught by Ting et al., in order to provide sufficient labeling for imaging in a living cell.

With respect to claims 7-8, Burbaum et al. teach the caging groups being covalently attached to the enzyme substrate, wherein the caging groups are photolabile and are removed by exposure to light of 366 nm (col. 22, lines 40-55), which is encompassed by the range of between about 60 nm and about 400 nm.

Regarding claims 9, 10 and 18, Ting et al. teach the first label and the substrate being physically connected (YFP and CFP are attached to the substrate peptide, pg. 15004,

Figure 1a), the substrate being a polypeptide (substrate is shown as a peptide, pg. 15004, Fig. 1a) and the enzyme being a protein kinase that phosphorylates serine/threonine and tyrosine (design works for serine/threonine and tyrosine kinases, pg. 15008, left column, second paragraph).

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the cell of Burbaum et al. in view of Walker et al., labels and signaling arrangements as taught by Ting et al., in order to study kinase and phosphatase functions, localization and activities inside living cells.

3. Claims 21, 23-25, 29-32, 304 and 305 are rejected under 35 U.S.C. 103(a) as being unpatentable over Burbaum et al. (US 5,981,207) in view of Walker et al. (Signaling pathways underlying eosinophil cell motility revealed by using caged peptides, 1998, PNAS, pg 1568-1573) further in view of Ting et al. (Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells, 2001, PNAS, Vol. 98, no. 26, pages 15003-15008) and Tsien et al. (US 6,900,304).

With respect to claims 21 and 29, Burbaum et al. in view of Walker et al. further in view of Ting et al., as applied to claims 1 and 18, teach the limitations of the claim. Ting et al. further teach the polypeptide comprising a second label wherein the first and second labels do not interact to produce the first signal when the substrate is not phosphorylated and a the first and second labels interact to produce a second signal when the substrate is phosphorylated (pg. 15004, Fig. 1a; pg. 15003, left column, last paragraph-right column, first paragraph). Ting et al. fail to teach the first and second labels interacting to produce a first signal when the substrate is not phosphorylated and the first and second not interacting upon phosphorylation to produce a second signal.

Tsien et al. teach a first and second label attached to a kinase substrate (col. 7, lines 16-20; col. 2, lines 14-32) and in a first embodiment the first and second label do not interact to produce a first signal when the substrate is not phosphorylated and the first and second label interacting to produce a second signal when the substrate is phosphorylated (Fig. 1A, col. 7, lines 29-31) and a second embodiment wherein the first and second label interact to produce a first signal when the substrate is not phosphorylated and upon phosphorylation the first and second label are prevented from interacting with each other (Fig. 1B, col. 7, lines 33-35), in order to detect kinase or phosphatase activity.

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the label of Burbaum et al. in view of Walker et al. further in view of Ting et al., a first and second label that are oriented such that the first and second label interact to produce a first signal when the substrate is not phosphorylated and upon phosphorylation the first and second label are prevented from interacting with each other as taught by Tsien et al. One having ordinary skill in the art would have been motivated to make such a change as a mere alternative and functionally equivalent detection technique and since the same detection of kinase activity would have been obtained. The use of alternative and functionally equivalent techniques would have been desirable to those of ordinary skill in the art based on the desired detection method and orientation required for a fluorescent signal.

Regarding claims 23 and 31, Ting et al. teach the first label located at the N-terminus of the polypeptide and the second label located at the C-terminus end of the polypeptide (pg. 15004, Fig. 1a).

With respect to claims 24 and 32, Ting et al. teach the first and second labels being fluorophores capable of exhibiting FRET (pg. 15003, left column, last paragraph-right column, first paragraph).

Regarding claim 25, Ting et al. teach the phosphorylation of the substrate triggers a conformational change in the polypeptide causing a FRET change between the first label and the second label (pg. 15003, left column).

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the composition of Burbaum et al. in view of Walker et al., labels, label placement and detection as taught by Ting et al., in order to provide fluorescent signal that is sufficient for real time imaging of enzyme activity.

4. Claims 13, 19, 22, 30 and 61 are rejected under 35 U.S.C. 103(a) as being unpatentable over Burbaum et al. (US 5,981,207) in view of Walker et al. (Signaling pathways underlying eosinophil cell motility revealed by using caged peptides, 1998, PNAS, pg 1568-1573) further in view of Ting et al. (Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells, 2001, PNAS, Vol. 98, no. 26, pages 15003-15008) and Tsien et al. (US 6,900,304), as applied to claims 1, 21 and 29, and Kris et al. (US 2003/0096232).

Burbaum et al. in view of Walker et al. further in view of Ting et al. and Tsien et al., teach a composition comprising an enzyme substrate, a first label and a first caging group, but fail to teach the substrate being specific for a protease and the location of the first label on the polypeptide.

Kris et al. teach detection of enzyme activity wherein a substrate is specific for a kinase or a protease (par. 18 and 78), in order to provide a surface that can detect the activity of a plurality of enzymes.

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the composition of Burbaum et al. in view of Walker et al. further in view of Ting et al. and Tsien et al., a protease as the enzyme as

taught by Kris et al., in order to identify potential blood thinners or agents which cause blood clots.

Regarding claim 19 and 22, Kris et al. also teach a polypeptide substrate (par. 18-19), wherein the one polypeptide comprises a first label and substrate for kinase (labeled antibodies bind to substrate, and therefore a single polypeptide comprises the substrate and first label, par. 256-258), the substrate comprising a tyrosine residue capable of being phosphorylated by the kinase (par. 256), wherein the first label is located at the tyrosine residue and exhibits a first signal when the residue is not phosphorylated and the second signal when the signal is phosphorylated (labels bind to phosphorylated substrates, and therefore bind to the phosphorylated residues, par. 258).

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to provide on the substrate of Burbaum et al. in view of Walker et al. further in view of Ting et al. and Tsien et al., placement of labels as taught by Kris et al., in order to accurately detect phosphorylation of the substrate.

With respect to claim 61, Ting et al. teach a kit comprising a substrate and a first label (col. 3, lines 16-23). Burbaum et al., as described above, teach a caging group, and Kris et al. teach including instructions for use in a kit (par. 84-87).

5. Claims 47-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Burbaum et al. in view of Walker et al. further in view of Ting et al., as applied to claim 1, and Fischer et al. (Cellular Delivery of Impermeable Effector Molecules in the Form of conjugates with Peptides capable of mediating membrane translocation, 2001, Bioconjugate Chemistry, Vol. 12, No. 6, pages 825-841).

Burbaum et al. in view of Walker et al. further in view of Ting et al., teach a sensor comprising one or more molecules, but fail to teach the one or more molecules associated with a cellular delivery module.

Fischer et al. teach delivery polypeptide vectors are used to transport entire proteins into a cell (pg. 827, right column, second paragraph), in order to provide delivery of proteins that are longer than a few peptides into a cell.

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the substrate of the composition of Ting et al. in view of Burbaum et al. further in view of Walker et al. and Ladner et al., a cellular delivery module of a polypeptide as taught by Fischer et al., in order to provide efficient preparation for in vivo analysis of enzyme activity.

Regarding claims 49 and 52-54, Fischer et al. teach the cellular delivery module covalently attached to the one or more molecules (pg. 825, abstract). Fischer et al. also teach that the cellular delivery module can also be used as a sub cellular delivery module by directing the proteins associated with the module to the same component (pg. 826, right column), in order to provide more accuracy. Fischer et al. teach the sub cellular delivery module being a polypeptide (pg. 827, right column, second paragraph) and covalently attached to the one or more molecules (pg. 825, abstract). It would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the composition of Burbaum et al. in view of Walker et al. further in view of Ting et al., cellular delivery module attached to one or more molecules or used as a sub cellular delivery module, in order to provide efficient preparation for in vivo analysis of enzyme activity and more accuracy.

Regarding claims 50, 51, 55 and 56, Burbaum et al. teach covalently attaching a caging group to a polypeptide in order to control activation of the polypeptide (col. 7, lines 37-47).

Allowable Subject Matter

6. Claims 26-28, 33-46, 306 and 307 are allowable over the prior art for the reasons stated in the office action dated 16 December 2005.

7. Claims 26-28 and 33-35 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Response to Arguments

8. Applicant's arguments filed 16 November 2007 have been fully considered but they are not persuasive. Applicant argues that there is no motivation to combine Burbaum with Ting and Walker because Ting et al. does not teach caging of an enzyme substrate. Applicant's argument is not persuasive because Ting et al. is not relied upon for teaching caging of a substrate. If Ting et al. taught caging of the substrate, the claims would be rejected under 35 USC 102(b) over Ting et al. Burbaum et al. is relied upon for teaching a caged and labeled enzyme substrate and Walker et al. is relied upon for injecting a caged peptide inside a cell. Ting et al. is relied upon only for teaching motivation of why one having ordinary skill in the art would want to put an enzyme substrate inside a cell (to detect and image cell activity). Applicant further argues that Burbaum designed substrates for secreted reporter enzymes and Walker focuses on control of protein activity, and there is no suggestion from Walker that other compounds should be injected into cells to detect activity inside a cell and therefore no motivation to combine references exists. However,

applicant's argument is not persuasive because Burbaum relied upon only for teaching the enzyme substrate being labeled and caged outside of a cell. Walker is relied upon only for injecting molecules into a cell, and Ting provides the motivation to place a particular enzyme substrate inside a cell.

9. Applicant further argues that the caged substrate of Burbaum and the caged substrates of Walker are chemically synthesized *in vitro*, while the protein constructs taught by Ting et al. are expressed *in vivo* and the protein constructs of Ting et al. are too large to be synthesized by current *in vitro* peptide synthesis techniques such as those used by Walker to produce caged peptides. These arguments have been considered and the rejections under 35 USC 103(a) over Ting, Burbaum, Walker and Ladner have been withdrawn.

10. Applicant's arguments with respect to claims 21 and 29 have been considered but are moot in view of the new ground(s) of rejection. Tsien et al. teach a that a FRET assay wherein the first and second labels interact to produce a first signal when the substrate is not phosphorylated and phosphorylation of the substrate prevents interaction of the first and second labels to product a second signal is functionally equivalent to the FRET assay taught by Ting et al.

Conclusion

Claims 26-28, 33-46, 306 and 307 are allowable over the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MELANIE YU whose telephone number is (571)272-2933. The examiner can normally be reached on M-F 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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